Supplementary Material for: TANGO: A Generic Tool for High-throughput 3D Image Analysis for Studying Nuclear Organization

Jean Ollion[1], Julien Cochennec[1], François Loll[1], Christophe Escudé[1], and Thomas Boudier[2]

[1]CNRS UMR7196, INSERM U565, MNHN, 37 rue Cuvier, 75005 Paris, France.
[2]Université Pierre et Marie Curie, IFR83/FRE3595, 7 quai St Bernard, 75005 Paris, France.

http ://biophysique.mnhn.fr/tango/

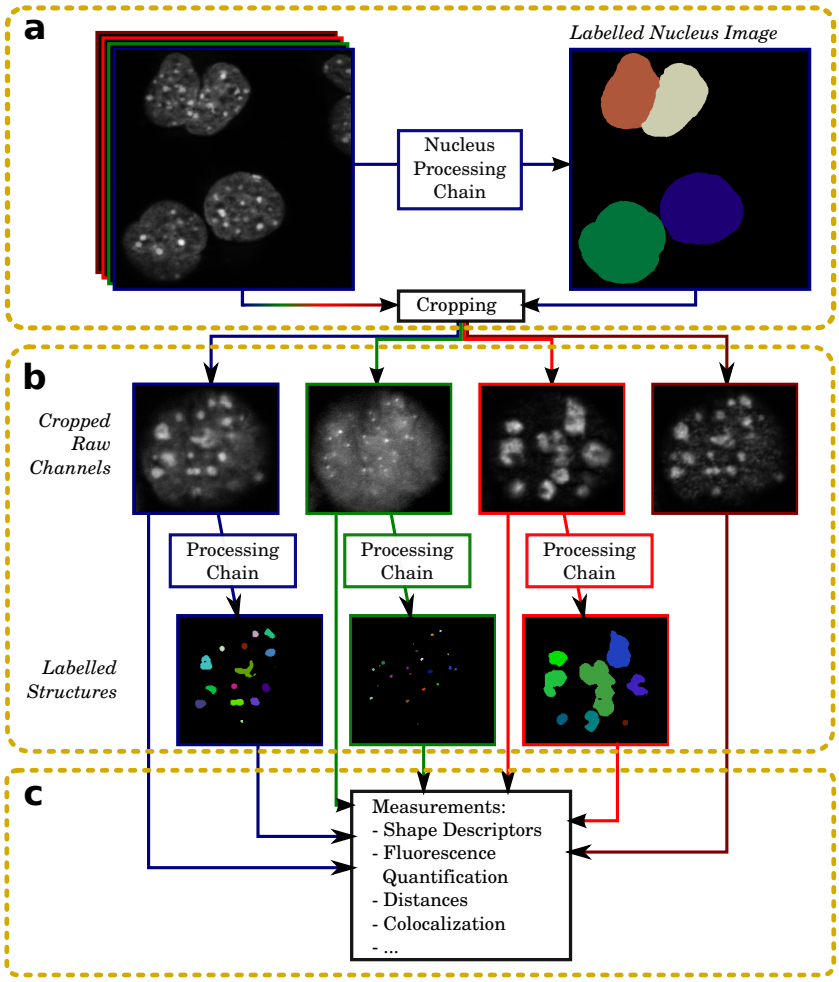

FIGURE S1 – Data processing workflow is composed of three main steps : (a) Segmentation of nuclei in each field, and cropping of channel images around the bounding box of each nucleus. (b) Segmentation of structures within each nucleus. (c) Quantitative image analysis in each nucleus, potentially using all raw channel images and labelled images.

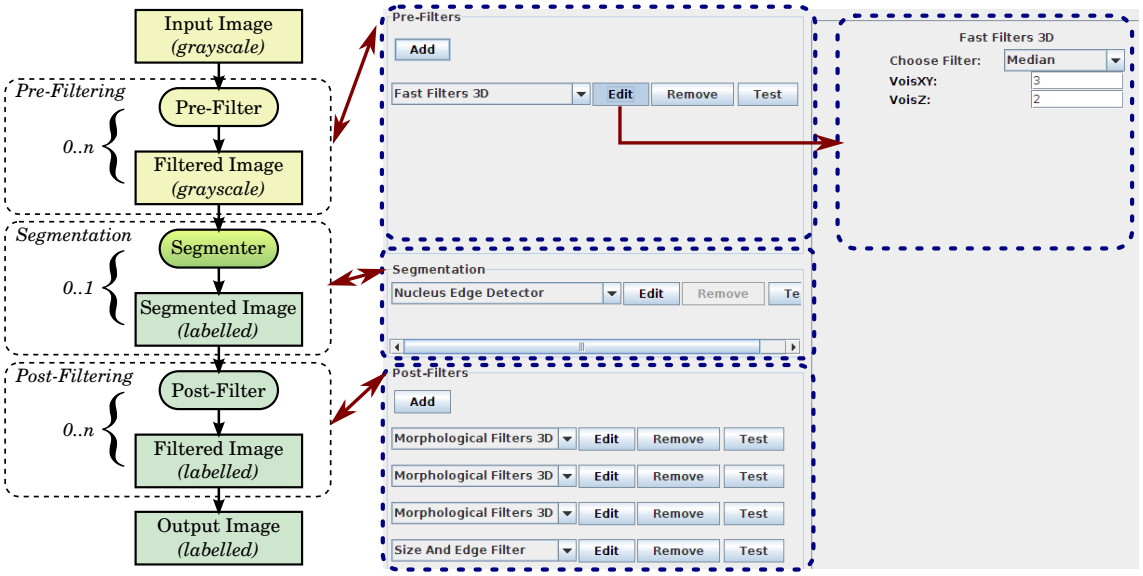

FIGURE S2 – Processing chains are composed of three steps : *pre-processing*, *segmentation* and *post-processing*. The *pre-processing* step is optional and can contain a sequence of several filters. The aim of this step is usually to correct common imaging artefacts, reduce noise (TANGO provides several filters, such as 3D-gaussian or 3D-median), and enhancing the signal to noise ratio (for instance, using 3D-top-hat filtering or 3D Laplacian of Gaussian). *Segmentation* is the process of identifying *objects* in the pre-processed image. Robust algorithms for segmentation of nuclei and of nuclear signals have been implemented, such as seed-based 3D watershed algorithm. The *post-processing* step is optional and can be composed of several filters. The aim of this step is to correct the segmented image. Classical operations are removing of small objects and morphological operations, such as closing. The right panel shows the processing chain editing interface in TANGO. For each of the three steps, processing operation can be chosen among operation of the corresponding category. If needed parameters can be adjusted (rightmost arrow). In order to help creation of processing chain, each operation can be tested individually on a selected nucleus (or on an opened image for nucleus segmentation).

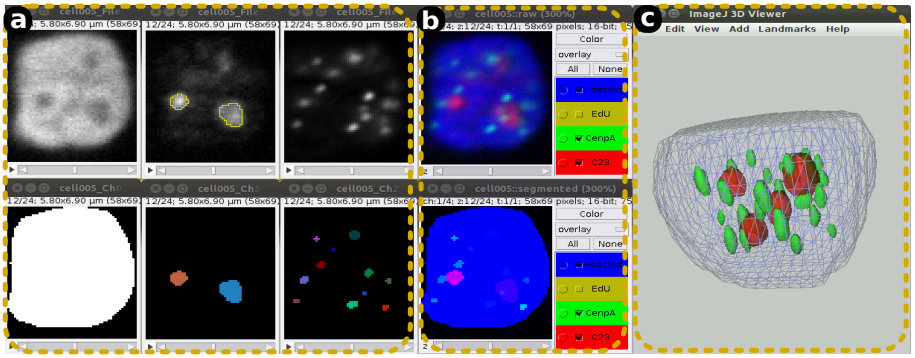

FIGURE S3 – Image Visualization. TANGO provides three modes of visualization for images : (a) standard ImageJ stack visualization, upper panel : grayscale images of different structures, lower panel : corresponding labelled images in which a color is assigned to each segmented object ; (b) overlay visualization of the images shown in (a) using the plug-in Image5d (10) ; (c) 3D representation of segmented structures using the plug-in ImageJ 3D Viewer (9). (a) and (b) . To assess the quality of segmentation, contours of segmented objects can be displayed on any opened image (as in (a), middle upper image) and will be automatically updated when changing the Z-plane.

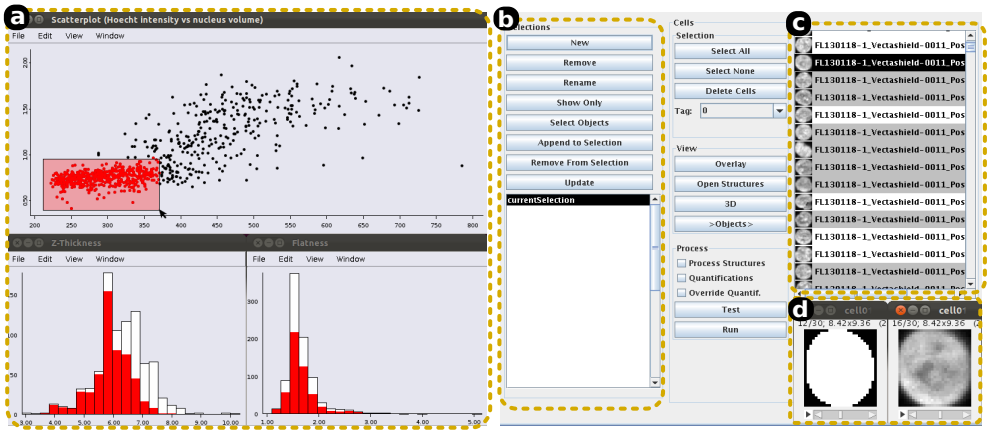

FIGURE S4 – Display and Analysis of sub-populations in R and TANGO. (a) Features from nuclei measured in TANGO can be imported in R using the r-package rtango, and plotted using the r-package Acinonyx (http ://rforge.net/Acinonyx/, package for interactive plots). In this case, each point of the scatter plot represents one nucleus. When a sub-population is selected, it is interactively reported on other plots. (b) Selections can be exported from R to TANGO, nuclei contained in the selection will be highlighted in the nucleus list (c) and the associated images can be visualized (d) or processed separately. Reciprocally, selections created or edited in TANGO are accessible within R, allowing one to analyse sub-populations of specific biological objects.

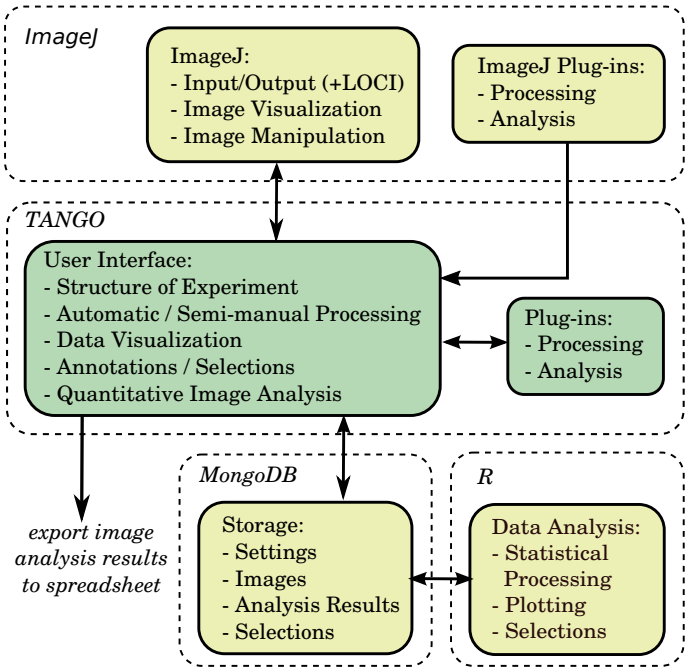

FIGURE S5 – TANGO is integrated inside two environments : ImageJ for image processing, analysis and visualization ; R for statistical analysis. All data are stored in a MongoDB database.

Name of module	Category	Short Description	Reference
Fast Filters 3D	Prefilter	3D filters (mean, median, tophat, etc)	Table S3.3
Histrogram Transformation		Operations on histogram (normalize, invert, scale, etc)	TANGO
Image Features		Features from image (gradient scale, hessian)	Table S3.
Misc 3D filters		3D filters (gaussian, bandpass, denoise, LoG3D)	Table S3.8,7,15,14
Translate 3D		Translation in x, y or z direction	Table S3.11
Simple Segmenter	Segmenter	Simple Thresholding	Adapted from Table S3.4
Hysteresis Segmenter		Segmentation using hysteresis thresholding (with two thresholds)	Adapted from Table S3.11
Nucleus Edge Detector		Nucleus segmentation based on edge detection, robust over intensity variations	TANGO
Seeded Watershed 3D		Watershed 3D seeded with regional minima	TANGO
Spot Detector 3D		seeded watershed 3D run on the maximum eigen value of the hessian transform, dedicated to the segmentation of small spots	TANGO
Spot Segmenter		Segment spots using seeds, watershed and local thresholding	Table S3.5
Erase Regions	PostFilter	Erase region based on intensity criteria (to use after Seeded Watershed 3D)	TANGO
Fast Filters 3D		3D filters (mean, median, tophat, etc)	Table S3.3
Merge Regions		Merge connected regions (to use after Seeded Watershed 3D)	TANGO
Morphological filters 3D		3D Morphological filters performed individually on objects (opening, closing, 2D/3D fill holes) optimized for speed for large radii (O(radius))	TANGO
Size and Edge Filters		Remove objects based on size or touching edges	TANGO
Watershed 2D		2D watershed to separate object in 2D images	Table S3.8
Re-label		Labels connected objects	TANGO
AutoThreshold	Thresholder	ImageJ's autothreshold methods. Computes a threshold based on histogram	Table S3.8
Kappa Sigma Clipping		Estimates the background value	Table S3.13
Percentage of bright pixels		Computes the threshold value corresponding to a percentage of brightest pixels in the image	TANGO
Threshold Adjustment		Adjust threshold computed with another method (add n*sigma, ...)	TANGO
Threshold operation		Operation between two thresholds computed with other methods (add, maximum, etc)	TANGO
Value		Constant value	TANGO

TABLE S1: Processing modules included in the first version of TANGO. Each module can contain several methods. See website for a more detailed description.

Name of module	Short Description	Reference
Distances	Center-center, center-border, border-border Distance between objects	TANGO
Grayscale spatial moments	3D Spatial Moments of signal	TANGO
JACOP colocalization	Colocalization based on intensity measurements	Table S3.12
Measure Geometrical Advanced	Measurements of 3D surfaces using meshes	Table S3.9
Measure Geometrical Simple	Geometrical measurements (volume, elongation, feret, etc)	Table S3.1
Minimal distances	Minimal distances between objects of two structures	TANGO
Object Colocalization	Estimates overlap between segmented objects (percentage of overlap)	Table S3.1
Shell Analysis	Normalized localization index in the nuclear space. Nucleus is divided into 3D layers of equal volume. Measures the index of the shell where the center of the object is located	TANGO
Eroded Volume Fraction	Continuous version of shell analysis	as described in Table S3.18
Signal quantification	Statistical descriptors of signal intensity within objects	Table S3.1
Signal quantification Layer	Statistical descriptors of signal intensity within a defined layer of objects (inside or outside)	TANGO
Texture	Classical matrix texture descriptor (entropy, etc)	Table S3.17

TABLE S2: Quantitative image analysis modules included in the first version of TANGO. Each module can contain several measurements. See website for a more detailed description.

1. 3D Analysis : http://imagejdocu.tudor.lu/doku.php?id=plugin:stacks:3d_roi_manager:start
2. 3D Euclidean Distance Map : http://www.optinav.com/Local_Thickness.htm
3. 3D Filters : http://imagejdocu.tudor.lu/doku.php?id=plugin:filter:3d_filters:start
4. 3D Object Counter : http://imagejdocu.tudor.lu/doku.php?id=plugin:analysis:3d_object_counter:start (reference, see 12)
5. 3D Spots Segmentation : http://imagejdocu.tudor.lu/doku.php?id=plugin:segmentation:3d_spots_segmentation:start
6. BoneJ : http://bonej.org/ (used for ROI Z-interpolation during the manual nucleus segmentation procedure)
7. DropletFinder : http://imagejdocu.tudor.lu/doku.php?id=plugin:analysis:droplet_counter:start
8. ImageJ http://rsbweb.nih.gov/ij/ Schneider CA, *et al.* (2012) NIH Image to ImageJ : 25 years of image analysis, *Nat. Methods*, **9**, 671-5
9. ImageJ 3D Viewer http://3dviewer.neurofly.de/. Schmid B., *et al.* (2010) A high-level 3D visualization API for Java and ImageJ, *BMC Bioinformatics*, **21**, 274
10. Image 5D http://developer.imagej.net/plugins/image5d
11. ImageScience : by Erik Meijering, http://www.imagescience.org/meijering/software/featurej/
12. JACOP : http://imagejdocu.tudor.lu/doku.php?id=plugin:analysis:jacop_2.0:just_another_colocalization_plugin:start Bolte S, *et al.* (2006) A guided tour into subcellular colocalization analysis in light microscopy, *J Microsc.*, **214**, 213-32
13. Kappa Sigma Clipping : http://hdl.handle.net/1926/367 Gaetan Lehmann (2006) Kappa Sigma Clipping *The insight Journal*
14. LoG3D (Laplacian of Gaussian) : http://bigwww.epfl.ch/sage/soft/LoG3D/ D. Sage, *et al.* (2005) Automatic Tracking of Individual Fluorescence Particles : Application to the Study of Chromosome Dynamics, *IEEE T. Image Proces.*, **14**, 1372-83
15. PureDenoise : http://bigwww.epfl.ch/algorithms/denoise/
16. R : cran.r-project.org. R Core Team, (2012) R : A language and environment for statistical computing, *R Foundation for Statistical Computing*
17. Texture analysis : http://rsbweb.nih.gov/ij/plugins/texture.html
18. Ballester, M. *et al.* (2008). The nuclear localization of WAP and CSN genes is modified by lactogenic hormones in HC11 cells.

TABLE S3: Third-party software used in TANGO and references